AD-A194 799 MICROWAVE-INDUCED CATARACTS OF THE EYE LENS: STRATEGIES FOR MODELLING AND..(U) UNIVERSITY OF WESTERN ONTARIO LONDON HEALTH SCIENCES CENTER.. J R TREVITHICK MAY 86 1/1

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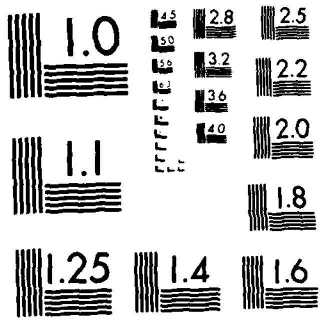

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AD-A194 799 REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Western Ontario, University of — 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Health Sciences Center, London, Ontario, Canada, N6A 5C1	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U.S. Army Medical Research & Development Command — 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: Contract No. DAMD17-83-C-3051
8c. ADDRESS (City, State, and ZIP Code): Frederick, Maryland 21701	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
62777A	3E1-62777A878	BB	016

11. TITLE (Include Security Classification)
Microwave-Induced Cataracts of the Eye Lens: Strategies for Modelling and Prevention in Vitro and In Vivo

12. PERSONAL AUTHOR(S)
John R. Trevithick

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual & Final	FROM 12/1/82 TO 4/30/86	1986 May	58

16. SUPPLEMENTARY NOTATION
Annual Report covers period of time January 1, 1985 through April 30, 1986
Final Report covers December 1, 1982 through April 30, 1986

17. COSATI CODES

FIELD	GROUP	SUB-GROUP
06	03	
06	18	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Cataracts, microwave radiation, membrane damage, globular degeneration, thermoelastic expansion, safety standards, radar

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

See attached summary

DTIC ELECTE MAY 23 1988 S D H

DISTRIBUTION STATEMENT A
Approved for public release; Distribution Unlimited

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT: ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Mrs. Virginia Miller	22b. TELEPHONE (Include Area Code): 301/663-7325 — 22c. OFFICE SYMBOL: SGRD-RMI-S

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

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MICROWAVE-INDUCED CATARACTS OF THE EYE LENS: STRATEGIES FOR MODELLING AND PREVENTION IN VITRO AND IN VIVO

Annual and Final Report

DR. JOHN R. TREVITHICK

May 1986

December 1, 1982 - April 30, 1986

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, Maryland 21701-5012

CONTRACT NO DAMD17-83-C-3051

University of Western Ontario
London, Ontario, Canada N6A 5C1

DOD DISTRIBUTION STATEMENT

Approved for Public Release: Distribution Unlimited

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Summary: Block 19

Over the period of this contract, the initial aims of this project were to develop techniques for incubating intact rat lenses *in vitro* in order to study the development of cataracts when lenses are exposed to CW and pulsed microwave irradiation. We planned to (1) establish minimum cataractogenic SARs for irradiation *in vitro* and (2) investigate the mechanisms of cataractogenesis in such lenses.

Initial studies (see final report June 1981 for DAMD17-80G-9449) had indicated a linear correlation between depth of cataractous globular degeneration and temperature when the lens was exposed to a short period of temperature elevation and postincubated for a period of 48 hr. This unexpected linear relationship was found between 37°C and 50°C; in addition, at 47°C and 50°C some very large globules were formed. Surprisingly, at a higher temperature (60°C) the lenses had normal opacity and acuity, apparently because they had been fixed by the high temperature. D-α-tocopherol acetate when added to lenses before incubation at 41°C, prevented most of the globular degeneration observed at this temperature.

In initial attempts to expose the lenses to microwaves, a system was devised to rapidly circulate thermostatted coolant around the lens while it was being irradiated. This system permitted experimental separation of heating effects in the lens from effects due to electromagnetic radiation, since there was no measureable temperature elevation in the lens with respect to the surrounding medium even at highest microwave exposure levels tested. Irradiation was performed for two exposure times and at three SAR values.

The results of the irradiation indicated that the effect of the electromagnetic radiation itself could be considered to be equivalent to heating, since at the highest dose rate and 37°C, large globules were formed, which would otherwise have been reported only at 47°C, equivalent to a temperature elevation of 10°C. Pulsed irradiation at high SAR values appeared to result in holes in the surface of cells, consistent with the idea that thermoacoustic expansion is causing mechanical damage to cell membranes. An estimate of amount of damage experienced as a result of total dose level of microwaves was consistent with the idea that the amount of damage is roughly proportional to the total dose delivered to the lens, and that a reciprocal relationship exists between dose rate and time required to cause a defined amount of globular degeneration.

More work under the contract (DAMD17-83C-3051) was done to explore the possibility of reciprocity, which has important implications for personnel who are chronically exposed to low levels of microwaves as well as those suffering from the effects of acute exposure. Initial studies on pulsed microwave (Exp. Eye Res. 40, 1-13 (1985)) indicated reciprocity between exposure duration and dose rate. Further studies have indicated that more of the variation in depth of damage could be explained by a model in which the effects of duration and SAR were separated. Nevertheless, the reciprocal effects model may provide an adequate fit for practical purposes and has the advantage of greater simplicity. For both models the pulsed irradiation produced 4.7 times the depth of damage caused by CW irradiation. This difference is consistent with a

previous CW-Pulsed comparison by Marha used in setting up the Czechoslovakian safety standard which sets different standards for pusled and CW irradiation (Marha, 1963).

The most recent work was done to compare the effects of varying pulse parameters likely to affect the pressure wave induced as a result of thermoelastic transduction. The operative pulse parameters to be studied were the pulse peak power and pulse duration. Work was done to explore these parameters under the contract extension until April 30, 1986. This work revealed significantly greater depth of damage at peak powers of 48 KW than with 24 KW peak power. The nature of the damage response to increases in average power and exposure time also was more pronounced. Deeper damage was observed at lower powers and exposure times for 48 KW pulse peak powers.

A more detailed analysis of the 48 KW data revealed significant increases in depth of damage associated with increased pulse duration, increased average power absorption and increased exposure time.

FORWORD

A. List of Professional Personnel Employed on This Project

Principal Investigator - Dr. John R. Trevithick, Ph.D.
Research Associate - Dr. Margaret O. Creighton, Ph.D.
Research Associate - Dr. P. Jill Stewart-DeHaan, Ph.D.
Research Associate - Dr. Madhu Sanwal, Ph.D.
Research Associate - Dr. Peter Galsworthy, Ph.D.

B. Animal Care

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Science - National Research Council, U.S.A.

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A. INTRODUCTION

Microwave cataractogenesis is generally considered to be a consequence of the average power absorbed in the ocular lens. It is often stated that temperature elevation alone produces lens pathology (e.g., Cleary, 1980). In fact, Kramar et al (1975) concluded that a retrolental temperature of 41°C is a threshold value for lenticular pathology (as determined by slit lamp examination) which may be associated with a SAR of ca 150 mW/g for ca 10^2 minutes of exposure. The results of Carpenter (1977) differed somewhat, since equivalent retrolental thermal histories produced by treatments using, either 2450 MHz, or direct conductive heating by a ring applied to the sclera, produced different lens pathology. Also incidence of lens pathology was reduced from 5/6 to 1/16 respectively. Similarly rabbit retrolental temperatures produced by restricting convective heat exchange via ear muffs and/or hot air directed to the ears, along with comparably reduced microwave dose, reduced the probability of lens opacity from 5/6 in the pure microwave case to 3/10 in the convection-restricted case. Carpenter concluded that retrolental temperature elevation is necessary but not sufficient to produce cataracts.

Our results (Stewart-DeHaan et al, 1983) from in vitro exposures (where precise temperature regulation was possible) differed from Carpenter in that murine lens histopathology produced by heat was not produced by CW power at 1 GHz. The effects produced by CW power also persisted in the absence of net temperature elevation when lens temperature was controlled by flow of coolant over the lens.

The issue of the thermal origins of lens histopathology may be considered from another direction. If heat is the only pertinent parameter, field modulation should have no effect so long as the absorbed average power, temperature and duration are closely controlled. Pilot results reported in 1980 (Stewart-DeHaan et al, 1980) suggested that pulsed irradiation optimized for thermoelastic expansion (TEE) produced lens histopathology which differed in both qualitative and quantitative terms from that produced by CW irradiation of the same average power. To the extent that these findings are correct, the average heating produced by the field cannot be the sole operant parameter.

Comparisons of pulsed and CW microwave irradiation have been performed in the past. Weiter (1975) measured lens ascorbic acid (a biochemical precursor of lens opacification) in rats after 48 hours incubation prior to exposure to pulsed and CW fields of 150 mW/cm^2 power density at the site where the rat was later to be placed for exposure. The frequency of irradiation was 2450 MHz, the pulses were 1 µS long at a frequency of 500 Hz. Heat bath, CW power and pulse power produced equivalent changes indicating little difference in effects. Birenbaum et al (1969) studied pulse and CW fields of the same average power at 5.5 GHz in rabbits. The pulse width was 10 µsec; the average power was 1 watt and the PRF was 500 Hz. The animals were exposed by a contact applicator consisting of a Stycast[1] dielectric lens and transition in close opposition to the conjunctive and cornea. A different assay, by slit lamp examination, failed to show any difference in threshold between CW and pulsed exposures (Carpenter, 1962). In these experiments Carpenter used 2450 MHz and

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[1] Trademark, Emerson & Cummings, Inc. Canton, MA.

9375 MHz: the pulsed exposures were at a 5% duty factor and an average power density of 140 mW/CM^2. Although the results were ambiguous, it has become increasingly frequent to cite this study as failing to show effects. One report of lens pathology from pulsed exposures (Richardson, 1951) has neither a CW comparison nor a suitable description of exposure conditions.

It has been shown that pulsed fields can elicit elastic waves in biological target organs: the potential of ocular hazards (Neelakartaswamy and Ramakrishnan, 1978, 1979) secondary to thermoelastic expansion has been cited as a new hazard mechanism, but no experimental evidence was presented.

The comparison of the extent of effects from pulsed and CW irradiation and the role of TEE as an additional mechanism for damage by pulsed microwave irradiation is important not only because of the generally held view that heating and associated temperature elevations are the only causative mechanisms of lens pathology. It also suggests the possibility of serious hazards arising from high peak power microwave emitters with low duty factors. In such situations the question arises if the hazard potential of the field is dependent only on average power of the field, or whether TEE-dependent effects may result in added hazards not presently appreciated. Since prior work has not fully addressed these issues in light of TEE theory we decided to perform the following experiments which determine effects of CW irradiation and to compare these results with previously reported results and additional new results for pulsed microwave irradiation at similar average powers.

In our previous (Stewart-DeHaan, et al, 1984) report, it was shown that the reciprocal model DEP = (POW X TIME)$^{.72}$ provided a good fit to the observed depth of damage to the ocular lens caused by pulsed wave irradiation. Our 1985 annual report presented results of a similar analysis comparing pulsed to continuous wave irradiation. In this report this data is repeated and models developed to incorporate results for both continous and pulsed waves, are further expanded to explore (i) effects of increasing peak pulse power and (ii) effects of changing pulse duration.

E. MATERIALS AND METHODS

After dissection, lenses of Sprague Dawley (Walter Reed strain) rats, 180-200 g average weight, with intact capsules, were either temporarily placed in phosphate-buffered saline in culture tubes or immediately transfered to the exposure cell described in a previous publication (Stewart-DeHaan, Creighton, Larsen, Jacobi, Ross, Sanwal, Guo, Guo and Trevithick, 1983) (Fig. 1) in which they were bathed by circulating phosphate-buffered saline (PBS) during exposure to microwave irradiation in WR975 wave guide at 918 MHz (Stewart-DeHaan et al, 1980, 1983). In lenses fixed immediately after irradiation, damage is observed in roughly a wedge shaped ring at the equator, with the apex of the wedge penetrating towards the nucleus. When damage occurred posteriorly and anteriorly it was found in a thin layer beginning at the capsule and proceeding inward towards the lens nucleus.

Lenses were irradiated, fixed immediately, then processed and examined either by scanning electron microscopy as we previously described (Stewart-DeHaan et al, 1983) or by light microscopy of 1 μ thick plastic sections of lenses, embedded in glycol methacrylate.

The average transmitted power was absorbed completely by the sample in the tuned waveguide system. Specific absorption rates for this system were measured previously without buffer circulating through the hoses connected to permit circulation of saline. Repeating this measurement, with saline not circulating but filling the hoses, using an microwavetransparent Luxtron probe to measure the temperature resulted in a decrease in SAR to approximately half that measured previously. With no saline in the hoses, the SAR values we previously reported were confirmed. The actual SAR values (for saline) at the location of the lens in the irradiation cell, with corresponding average transmitted powers, were: power (SAR) 2 W (23 mW/g), 6 W (69 mW/g), 20 W (231 mW/g), 65 W (750 mW/g). For comparison, those we previously reported (and confirmed) were 2 W (40 mW/g), 6 W (120 mW/g), 20 W (400 mW/g) and 65 W (1.3 W/g). The pertinent formula for calculation of the SAR, modified by Lin et al, 1978, from the original formula of Johnson and Guy (1972) (SAR (w/cc) = 4.18 c T/t), to permit expression in W/g instead of W/cc, is SAR (W/kg) = 4.18 c T/t, where c is the specific heat of the sample in cal deg^{-1} g^{-1}, ρ is the density in g/cc, 4.18 is a constant for unit conversion (1 calorie = 4.18 joules), and T is the temperature elevation in $^{\circ}$C during the exposure duration t in seconds. For the calculation we assumed the specific heat of water to be 0.998 cal deg^{-1} g^{-1} and the density 0.998 g/cc for saline. Experiments are in progress to determine the actual values for heating.

Five separate conditions, at different times and average powers to permit the same total energy to be delivered during 6, 20 or 60 minutes of irradiation, at different SAR values, 11.5, 23, 69, 231 mW/g and 750 W/g, were examined for both CW and pulsed microwave: 0.23 watt-min/g, 0.46 watt-min/g, 1.31 watt-min/g, 6.9 watt-min/g, and a maximum of 15 watt-min/g.

Microwave irradiation was delivered to the lens in the pulsed mode with [illegible], 10 μsec and 20 μsec pulse width and 24 KW and 48 KW of peak transmitted power. Repetition rates were varied to obtain the various average powers.

For each lens in which damage could be observed, granular degeneration occurred in a depressed ring in the zonular region and around the lens equator, with the apex of the depression towards the lens nucleus (see Results). The maximum depth of degeneration was measured at the deepest penetration of the damage. Measurement was made in the region 40-60 μm immediately posterior to the centre of the zonular attachment to the exterior of the capsule. The measurement was made from the inside of the lens capsule to the assessed maximum depth of visible damage. The two determinations, (one on each side of the lens) were averaged (since they were usually quite similar and that average represented an observation in the analysis. If only one determination was possible, this determination alone was used. This occurred only in three lenses, all being exposed to pulsed waves for radiation. These values were used to determine the average of maximum depths of damage for the particular condition chosen. All determinations were performed blind using a numerical code and the code broken after the analyses were complete.

C. STATISTICAL ANALYSES

C. 1 Initial Pulsed-CW Comparisons

An analysis of the effects of pulsed vs continuous wave irradiation on the ocular lens was performed. The purpose of the analyses was to compare the depth of granular degeneration (DEP) produced by continuous and pulsed wave irradiation at various dose rates (POW) and lengths of exposure (TIME).

A one-way analysis of variance (ANOVA) was performed on the continuous wave data alone and the means for the 11 combinations of POW X TIME compared using the modified Tukey method in multiple comparisons (1953). Such an analysis has been reported previously on pulsed microwave irradiation (Stewart-DeHaan, 1985).

In order to test reciprocity between duration of exposure and dose rate, overall, two alternative models fit to continuous wave and pulsed data were used:

$$(1) \quad DEP = b_o\,(POW \times TIME)^{b_1}\,e_1$$

$$(2) \quad DEP = b_o\,(POW)^{b_1}\,(TIME)^{b_2}\,e_2$$

The first model states that POW and TIME act in a reciprocal fashion to determine the depth of damage and is referred to as the reciprocal effects model. Model (2) allows for the possibility of separate effects of POW and TIME through coefficients b_1 and b_2. In both models e represents a random multiplicative disturbance. Overall comparison of pulsed and CW effects was conveniently performed, using these models, for both sets of data.

The adequacy of model (1) was tested against the alternative expressed by model (2). Since both models were log-linear this was done by fitting the transformed models

$$(1) \quad \ln(DEP) = \ln b_o + b_1 \ln(POW \times TIME) + \ln e_1$$

$$(2) \quad \ln(DEP) = \ln b_o + b_1 \ln(POW) + b_2 \ln(TIME) + \ln e_2$$

by multiple regression. It was assumed that $\ln e_1$ and $\ln e_2$ were normally distributed with mean zero and variances σ_1^2 and σ_2^2 respectively.

Inspection of the results in (1) suggested that the depth of damage produced by the two radiation types differed by a multiplicative constant. Thus the reciprocal model

$$(3)\quad DEP = b_o b_1^x\ (POW\ X\ TIME)^{b_2} e$$

was proposed, where x = 0 for continuous waves and x = 1 for pulsed waves.

$$(4)\quad DEP = b_o b_1\ (POW)^{b_2} (TIME)^{b_3} e.$$

The linear forms of these models were:

$$(3)'\quad \ln(DEP) = \ln b_o + x \ln b_1 + b_2 \ln(POW\ X\ TIME) + \ln e$$

$$(4)'\quad \ln(DEP) = \ln b_o + x \ln b_1 + b_2 \ln(POW) + b_3 \ln(TIME) + \ln e.$$

These models were fit to the combined data using multiple linear regression.

A second one-way ANOVA was performed including data for pulsed and continuous wave irradiation at each of 10 POW X TIME combinations. The method of contrasts was then used to compare the two means at each combination of POW X TIME.

C.2 Most Recent Pulsed-CW comparison

C.2.1 Comparison of 24 and 48 KW Series at 10 μs

In (1) the comparison of 24 and 48 KW peak powers was confounded with different pulse widths (10 and 20 μs). In this analysis of the new data from the 48 KW series at 10 μs was used so that the effects of changing peak power alone could be examined. There was, however, another complication in this comparison being that the 24 and 48 KW series were done with a different configuration of the apparatus with the result of different actual absorption of the pulse emitted (POW). To account for this in the analysis the actual power absorbed (NPOW = .4375 x POW, for 48 KW data) was used as a covariate in analysis of covariance (ANCOA). Thus the comparison of 24 and 48 KW means is a comparison of means adjusted for the discrepancy in absorbed irradiation. ANCOVA makes this adjustment based on the observed correlation between NPOW and depth of damage.

This analysis was performed with the subprogram ANOVA from SPSS (2).

C.2.2 Reciprocal and Separate Effects of Exposure Time and Average Power at 48 KW and 10 μs

Again this analysis was similar to that contained in (1) using data for 10 μs to yield a more direct comparison with the results from 24 KW at 10 μs which were:

(1) $DEP \sim (POW \times TIME)^{.72}, \; R^2 = .78$

(2) $DEP \sim (POW)^{.67}(TIME)^{.83}, \; R^2 = .79$

The values used for average power where the amounts actually absorbed (NPOW) to make the coefficients comparable.

C. 4 Revised Comparison of Previous and New 24 KW Results

This analysis consisted of fitting the 12 observations from the new series to the separate and reciprocal effects models with POW replaced by NPOW. The coefficients of the resulting models were tested against those given in the section above.

C. 5 Three Factor ANOVA

Observations taken at 2, 10 and 20 μs pulse widths were combined in an analysis of variance (ANOVA) for the 4 x 3 x 3 (POW X TIME X PW) factorial design. This analysis was also performed using the ANOVA subprogram in SPSS (Version 9.0) (2). In this analysis the main effects of each factor were assessed with the other two factors held constant. Similarly the two and three-way interactions were assessed with all main effects and other interactions of the same or lower order held constant. This means, for example, that the test for the main effect of PW was based on the variation attributable to that factor after the variation attributable to POW and TIME had been accounted for.

C. 6 Three Factor Regression

Two regression models were fit to the complete set of observations taken at 48 KW. The first allowed for reciprocal effects of POW and TIME with [illegible] effect of PW. The second provided for separate estimates of the log-linear effects of these factors. The actual amount of irradiation absorbed (NPOW) was used to make the results comparable to previous models.

D. RESULTS

D. 1 Lens Damage Observed with Pulsed and CW Microwave

For lenses fixed immediately after exposure the damage observed was of several types: (1) holes within the fiber cells, especially in the region of zonular attachment in the equatorial region, (2) capsular effects, such as pitting or surface granulation, (3) globular degeneration, sometimes covering large subcapsular regions but mainly within the zonular attachment and equatorial region, (4) foam, located immediately subcapsularly within the same region and (5) granulation of fiber cells, which can extend deep within the lens (Stewart-DeHaan, 1985). Incubation of the lens for a further 48 hr. as previously reported (Stewart-DeHaan et al. 1983, 1985) results in more obvious damage as effects on cell membrane integrity are expressed in more advanced damage: globular degeneration and foam.

Although some damage was seen in lenses fixed immediately after exposure to pulsed microwaves, the threshold at which damage was observed in 50% of the lenses (TD_{50}) varied depending on the type of damage (Table 1). For lenses exposed to CW waves, not all types of damage were seen; usually the TD_{50} occurred at higher powers than the TD_{50} observed for pulsed microwaves (Table 1).

Except for the similar effects observed for both pulsed and CW irradiation after 60 minutes exposure to 1/2 W (10 mW/g) average power, the lowest power at which damage was observed occurred at lower average powers for pulsed irradiation than for CW. Conversely, when compared only by ANOVA, for the same average SAR (or power) x time combinations, the damage observed following pulsed irradiation was always greater than for CW, and at higher average powers the damage was much more extensive. For instance, at 65 W, the damage from pulsed irradiation was extensive: irregular, jagged areas of granular degeneration intruded deep into the cortex, while the CW irradiation only resulted in small areas of granular degeneration at the lens equator. A diagrammatic view of changes observed in the area of the lens equator after pulsed and CW irradiation different durations and SAR values is seen in Fig. 2-4. In figures 2 and 3, CW (Figure 2) and pulsed (Fig. 3) irradiation effects are represented diagrammatically after exposure for the same lengths of time 6 min, 20 min and 60 min. In Figure 4, at the threshold value for the first observable damage at the lens equator (for either pulsed or CW), this damage may be compared to the amount of damage shown by CW or pulsed, respectively. For instance in Fig. 4c the lowest pulsed power level at which damage is observed is compared to Fig. 4d, which illustrates the absence of any comparable damage after the same CW power level and time. The more extensive damage caused by pulsed irradiation (Fig. 4e) at the CW threshold exposure (Fig. 4f) (20 W, SAR 230 mW/g) is compared in Fig. 4e and 4f. Finally the [illegible]ve depth of damage is compared for pulsed (Fig. 4g) and CW (Fig. 4h), resulting in a ratio of pulsed/CW damage of approximately 3.0 at the maximum SAR (65 W SAR 750 mW/g).

For irradiation at 20 minutes, the lowest power (Fig. 5c) at which the pulsed irradiation caused discernable damage was 1/2 W (SAR 518 mW/g). At this average power, no damage was apparent in the CW-irradiated sample at the same power. At the lowest CW power at which damage could be observed (2 W, SAR 23 mW/g) (Fig. 5c) the pulsed irradiation caused significantly more damage (30 m deep). The maximum damage, at 65W (SAR 750 mW/g) (Fig. 5g) is observed to be approximately four times as deep for pulsed, as compared to CW, irradiation (Fig. 5h).

Following 60 minutes irradiation (Fig. [illegible]), the minimum damage occurred for both pulsed (Fig. 6c) and CW (Fig. 6d) irradiation at 1/2 W average power (SAR 518 mW/g), although slightly more damage occurred with pulsed than with CW irradiation. At an intermediate power level (Fig. 6e, f, SAR 69 mW/g), the pulsed irradiation caused damage to approximately 3.3 times as great a depth as the CW irradiation at the same average power (50 m vs 15 m). At the maximum average power (65 W, 750 mW/g) (Fig. 6g, h) the ratio of depth of damage for pulsed/CW irradiated samples was approximately 1.5.

Statistical evaluation of the data was performed to evaluate the data shown in Table 2. As in the previous results for pulsed irradiation (Stewart-DeHaan et al, 1984), the continuous wave irradiation produced increasing depth

of damage with increasing dose rate and increasing time of exposure (Fig. 5). Pulsed wave irradiation produced consistently greater depth of damage than did continuous wave irradiation except at the 2 watt 6 minute combination. The pulsed CW differences were subjected to detailed comparison at 10 different combinations of POW X TIME.

D. 2 Models for Continuous Wave Irradiation

The estimated models (1)' and (2)' are shown in Table 3. The means for the data used are given in Table 2. The separate effects model, DEP = $0.4(\mathrm{POW})^{.82}(\mathrm{TIME})^{1.22}$, accounted for 76% of the observed variation in DEP while the reciprocal mode, DEP = $.10(\mathrm{POW\ X\ TIME})^{.88}$, accounted for 67% of this variation. The F test shown in Table 4 showed that for the continuous wave data the separate effects model explained significantly more of the observed variation in DEP.

The 95% confidence intervals for the parameters estimated in models (1) and (2) are given in Table 5.

D. 3 Models for the Combined Data for 24 KW, 10 μsec pulses compared to CW

The fitted equations for models (3)' and (4)' are given in Table 6. The separate effects model DEP = $.09(4.66)\mathrm{x}(\mathrm{POW})^{.74}(\mathrm{TIME})^{.97}$, explained slightly more of the variation in depth of damage than the reciprocal effects model, DEP = $.15(4.71)\mathrm{x}(\mathrm{POW\ X\ TIME})^{.78}$. Table 7 shows that this small difference was significant or the separate effects model explained significantly more of the observed variation in depth. The means for the additional pulsed wave data used in this analysis are given in Table 8.

The 95% confidence intervals for the parameters estimated in models (3) and (4) are given in Table 9.

E. DISCUSSION

E. 1 CW Compared to Pulsed Irradiation (24 KW, 10 μsec pulses)

The thermogenesis of ocular lens histopathology remains an issue of importance in microwave biomedical research. Its importance derives from two factors: the need for improved biophysical understanding of the mechanism of histopathologic effects secondary to microwave exposure, and the need to insure safety under conditions of exposure to high peak power microwave emissions where the average power absorbed may not fully represent the hazard.

The results described here clearly indicate that for microwave irradiation at 918 mHz modulation of the signal to produce 10 μsec pulses results in more damage at the same average power for every combination tested except one (2 W SAR of 23 mW/g for 6 min). Although the separate effects models explained significantly more of the variation in depth of damage, the reciprocal models may provide an adequate fit for practical purposes with the advantage of greater simplicity. This is particuarly true in the case of the combined data where the actual difference in R^2 may be of little practical importance but was significant because the large number of observations produced a very powerful test.

The graphs of both models, showing their fit to the 24 KW peak pulse power data, are given in Figure 7 and 8. For both models 3 and 4 the pulsed irradiation produces 4.7 x the depth of damage caused by the CW mode. Of the several mechanisms by which such additional modulation-dependent damage could occur, (viz. thermoelastic expansion (TEE), electrostriction effects (ESE) etc. the most likely mechanism is TEE, resulting in pressure waves induced in the aqueous medium and lens tissue by thermoacoustic expansion following each pulse of microwave energy. The acoustic measurements previously performed and reported elsewhere (Guo, Guo and Larsen, 1984) are consistent with such a mechanism: pressure waves are induced by each pulse. These are capable of causing the additional types of physical damage previously noted for pulsed microwaves - holes in cell membranes, large globules at higher power etc. This work thus provides evidence for significantly increased damage as a result of signal modulation at the same average power and wavelength. The work of Marha (1963) on biological damage by CW and pulsed microwaves used to irradiate whole rats supports the idea that high energy pulsed irradiation causes significantly more biological damage even at the same average power of irradiation. Our findings confirm this in indicating approximately 4-5 times greater damage for pulsed wave irradiation as compared to CW irradiation. Although the irradiation pulses were only 2 μsec duration they would have been able to cause a significant thermoacoustic effect. For this reason we continued to compare pulses of different duration and peak powers.

F. RESULTS

F. 1 Comparison of 24 and 48 KW Series at 10 μsec

Tables 10 and 11 show the data used in this analysis. The results of ANCOVA are given in Table 12. The absorbed irradiation (NPOW) had a significant positive correlation with depth of damage. When peak power means were adjusted for the discrepancy in absorbed power the result was that 48 KW produced significantly deeper damage ($p = .02$). Greater time of exposure also produced significantly more damage ($p = .001$). The significant PP X TIME interaction indicated that the effect of peak power varied from one exposure time to another. Since the adjusted means were not broken down by PP X TIME it was not possible to comment on the nature of this variation.

F. 2 Reciprocal and Separate Effects of Exposure at 48 KW and 10 μsec

The reciprocal and separate effects models obtained from the 48 KW results at 10 μsec were:

a) $DEP = 8.58\,(POW \times TIME)^{[illegible]}$, $R^2 = .55$

b) DEP = [illegible] (POW)[illegible] (TIME)[illegible], R^2 = [illegible]

Neither model showed as good an overall fit to the 48 KW data as it did to the 24 KW data in terms of the coefficient of determination (R^2). As for the 24 KW data there was little to choose between the reciprocal and separate effects models on these grounds (.55 vs .56). Both models contained positive multiplicative constants with smaller effects of POW and TIME. This would indicate greater depth of damage at lower levels of POW and TIME that dampened

out more quickly than for the 24 KW results. This is illustrated in the damage by power profiles of Figure 9. However, these results must be interpreted with some caution due to the lack of observations for high levels of absorbed irradiation in the 48 KW series.

The coefficients in these models were all statistically significant ($p < .001$), showing a significant log-linear relationship of depth with POW or TIME or (POW X TIME).

F. 3 Revised Comparison of Previous and New 24 KW Results

Table 13 gives the data from the series run on the new apparatus at 24 KW. When this data was fit to the reciprocal and separate effects models with average power adjusted to reflect the amount of irradiation actually absorbed the results were:

1) $DEP = 8.17\ (POW \times TIME)^{.41},\ R^2 = .82$

2) $DEP = 6.89\ (POW)^{.37}(TIME)^{.49},\ R^2 = .84$

These regression functions showed some evidence of change from the original series ($p = .06$ and $p = .11$, respectively).

F. 4 Analysis of the Complete 48 KW Data

F.4.1 Three Factor ANOVA

Tables 14, 15 and 16 give the mean depth of damage at 48 KW for pulse widths of 2, 10, and 20 μsec. broken down by average power and time. The results of the three factor ANOVA are summarized in Table 17 and illustrated in Figures 10-12.

Depth of damage increased significantly with increases in each of the three factors. Figure 10 shows that the damage profiles were not parallel when examined by POW and PW. This accounts for the significant PW X POW interaction ($p = .001$). Figure 11 shows the situation for PW X TIME with the lack of increase from 10 to 20 μsec with a 6 minute exposure time being largely responsible for the significant interaction ($p = .001$). The relatively parallel profiles of damage by power and time in Figure 12 explain the lack of a POW X TIME interaction ($p = .79$). The significant three way interaction indicates that each two-way profile would differ if plotted for each level of the third factor.

F.4.2 Three Factor Regressions

The following results were obtained from regression analysis as described in Section 2.4.2.

1) $DEP = 13.6\ (POW \times TIME)^{.35}(PW)^{-.09};\ R^2 = .47$

2) $DEP = 12.2\ (POW)^{.35}(TIME)^{.37}(PW)^{-.09};\ R^2 = .47$

The coefficients (exponents) of POW, TIME, or their product were significant in both models ($p < .001$) as were the constant multipliers ($p < .001$). The coefficient of PW was not significantly different from zero ($p = .01$) in either model and this term could be dropped.

The proportion of variation explained (R^2) was not high for either model. The value of R^2 for the ANOVA model discussed in Section F.4.1 was considerably higher (.70). This was due to the fact that the regression model did not provide for interactions between all the factors. Such interactions explained a significant amount of variation in ANOVA. This also accounts for the fact that the effect of PW was significant in ANOVA but not in these particular regression models.

F. 5 Prior Work and Its Interpretation

Prior work has been interpreted to reach the conclusion that exposure to pulsed CW irradiation of the same average power results in a similar degree of damage for biosystems. It has been felt that this danger is largely, if not solely, the result of average temperature elevation (Cleary, 1980). The historical base on which this conclusion is reached can be interpreted differently, especially in light of thermoelastic expansion theory. Even so, Carpenter (1966) remains skeptical that pulsed and CW fields are equivalent, but his data are not sufficently clear to reach a firm conclusion. In view of the TEE mechanism, Carptenter's pulse exposures were done at an extraordinarily low duty factor (50%) at an average power density of 140 mW/cm^2. Thus, the pulse width was much too long to produce TEE efficiently, according to equations developed to calculate the thermoacoustic transduction (Lin, 1978). The absence of SAR data and the coupling iris used for the X-band pulse exposures make interpretation of this study difficult.

The work of Birenbaum et al (1969) is more often cited as evidence for the equivalance of pulse and CW effects. This experiment was done with a series of 100 rabbits exposed to pulsed microwaves and 62 exposed to CW. Birenbaum et al used a variation of a contact applicator method devised by Carpenter (1968). The Birenbaum applicator consisted of a section of dominant model "C" band rectangular guide (WR187) followed by a transition to double ridged guide with Stycast (ϵ_r = 12, tan d = 0.001) dielectric loading between the ridges. This was followed by a transition to circular guide completely filled with the Stycast dielectric. This final section was [illegible] in diameter, 0.458" (1.23 cm) long and machined for a concave [illegible] hemisphere into which the animal's eye was placed. The eye lid was sutured open and a pseudo tear film layer was applied to the conjunctive cornea. The exposures were performed at two average powers: 1 watt and 1.2 watt. No differences were detected between pulse and CW exposures at the same average power for 5 µsec pulses at a 10^{-3} duty factor. This suggests that the peak energy was 5 mJ over an aperture of 1.23 cm^2 to result in a peak energy

density of about 4 mJ/cm^2. This should have been a sufficient energy density and reasonably efficient pulse width for the frequency of operation to achieve TEE. Yet no differential effect was observed.

The reason that no differential effect was observed may have been a consequence of the applicator and the biological end points. Firstly, the applicator prevented normal evaporative cooling of the cornea. Secondly, the applicator was likely to be at an elevated temperature. Based on a tan d of 0.001 and e' = 12, the e''_r was 1.2×10^{-2}. The SAR of the Stycast dielectric may be estimated from SAR = $(0.556 \times 10^{-12})\, e''_r\, 1\, E^2$ of (watts/cm^3) which yields approximately 1.8×10^{-3} watt/cm^3. The volume of the end piece was ca 1.4/cm^3 and its density is 2.24 g/cm^3. Given the relationship between SAR and temperature elevation per unit time SAR = 4.186 $\rho cT/t$ (Johnson and Guy, 1972; Stewart-DeHaan et al., 1983) where ρ is the density in g/cc and c is the specific heat and based on a specific heat in the order of unity, the temperature rise would be ca 1/8°C sec. The exposure duration was 3 min which would give an end temperature of the front applicator surface (ignoring heat transfer to the guide) of ca 20°C. This added to base line temperature of 23°C and allowing complete cooling between runs would place the applicator at ca 43°C directly on the conjunctiva/cornea. This is a sufficient thermal insult to dominate any other aspect of the exposure. In fact, there is ample evidence cited in Birenbaum's results of acute anterior chamber effects in both the pulse and CW groups. This includes "acute, inflammitory reactions of the cornea. conjunctiva, iris and/or ciliary body were observed in many and probably produced in <u>every</u> exposed rabbit eye". These reactions were "frequently severe" and has "usually subsided by the fourth day".

Additional evidence of a primary anterior chamber effect due to corneal heating and thermal diffusion to the lens is the observation that lens opacity was unusually long in latency (2 to 3 weeks post exposure) and "was present in the anterior portion of the lens". This is unusual with respect to the latency. Carpenter (1977) observed absolutely no effects until after 24-48 hours and opacity after 3 to 7 days. The anterior location of the lens injury is also unusual. The 5.5 GHz operating frequency was not high enough to produce preferential anterior chamber heating (Richardson 1951 and unpublished observations of LEL). The expected site of maximal intra-ocular temperature elevation at 5.5 GHs is retrolental and the expected opacity would be posterior subcapsular. This strongly suggests that Birenbaum's results are caused by corneal heating via conduction from a hot applicator.

Pulsed fields have been implicated in ultrastructural damage to cell membranes and mitochondria with <u>in vitro</u> exposures of neuroblastoma cells in culture (Webber et al. 1980). The exposures took place at 2.7 GHz with 1 μsec pulse width and 330 Hz PRF. Cell membrane breaks and myelin figures in mitochondria were observed. These could not be duplicated by simple heating to equivalent or higher temperatures.

No comparisons were made to effects caused by CW irradiations but the effect of the heat bath treatment is in contrast to the RMS value of the field. No mention was made of TEE, and the pulse width would be suboptimal for the frequency of operation and target dielectric; nevertheless, TEE could be implicated.

The mechanism not implicated in the contrast of pulse with CW exposure is pearl chain formation. This is an electrostatic effect which causes orientation of dipole objects along force lines during a pulse. In between pulses, Brownian motion disorders the pearl chains. Sher et al (1970) clearly demonstrate that pearl chain formation is not augmented in pulsed fields even though the peak values are higher than the equivalent power implied by static field.

REFERENCES

BIRENBAUM, L. GROSOF, G.M., ROSENTHAL, S.W. & SAPET, M.M. ([illegible]). IEEE Transactions on Bio-Medical Engineering 16, 7-14.

BROWN, P.V. & WYETH, N.C. (1983). Rev. Sci. Instrum. 54, 85-89.

CARPENTER, R.L., BIDDLE, D.K. & VAN UMMERSEN, C.A. (1960). IRE Trans. on medical Electronics Vol. ME7, 152-157.

CARPENTER, R.L. (1962). USAF AF 41 (657-06 12 ADC-TDR-62-131.

CARPENTER, R.L. & CLARK, V.A. (1966). Responses to Radio-frequency Radiation. IN: "Environmental Biology" (Altman, P.L. and Dittmer, D.S. Ed.), DS. Bethesda, MD, pp. 131-138.

CARPENTER, R.L. (1968). J. Microwave Power 3, 3-19.

CARPENTER, R.L., HAGAN, G.J. & FERRI, E.S. (1975). Ann. N.Y. Acad. Sci. 247, 142-154.

CARPENTER, R.L., HAGEN, G.J. & DONOVAN, G.L. (1977). Are Cataracts Thermally Caused? IN: "Symposium on Biological Effects and Measurements of Radio Frequency/Microwaves". Rockville, pp. 352-379.

CLEARY, S.F. (1980). Proceeding of the IEEE 68, 49-55.

GUO, T.C., GUO, W.W. & LARSEN, L.E. (1984). IEEE Transactions 32, 835-843.

JOHNSON, C.C. & GUY, A.W. (1972). Proc. IEEE 60, 692-718.

KRAMAR, P.O., EMERY, A.F., BUY, A.W. & LIN, J.C. (1975). Ann. N.Y. Acad. Sci. 247, 155-164.

LIN, J.C. (1978). Microwave Auditory Effects and Applications. Thomas Springfield Ill.

MARHA, K. (1963). Pracovni Lekarstvi 15, No. 5, 238 (in Czech). English translation NIOSH 10-332.

NEELAKANTASWAMY, P.S. & RAMAKRISHNAN, K.P. (1978). Biomedizinische Technik. 23, 109-113.

NEELAKANTASWAMY, P.S. & RAMAKRISHNAN, K.P. (1979). J. Biochemanics 12, 205-210.

[illegible] N.H. et al (1975). Statistical Package for the Social Sciences. Second edition. McGraw-Hill Book Company, New York, p. 428.

[illegible]LSON, A.W., DUANE, T.D. & HINES, H.M. (1951). A.M.A. Archives of Ophthalmology 45, 382-386.

[illegible] D., KRESCH, E. & SCHWAN, H.P. (1970). Biophysical Journal 10, 970-979.

[illegible]-DEHAAN, P.J., CREIGHTON, M.O., LARSEN, L.E., JACOBI, J.H., ROSS, W.M. & TREVITHICK, J.R. (1980). IEEE M.T.T.S. International Microwave Symposium Digest, pp. 341-345.

STEWART-DEHAAN, P.J., CREIGHTON, M.O., SANWAL, M., ROSS, W.M. & TREVITHICK, J.R. (1981). Ex. Eye Res. 32, 51-60.

[illegible]-DEHAAN, P.J., CREIGHTON, M.O., LARSEN, L.E., JACOBI, J.H., ROSS, W.M., SANWAL, M., GUO, T.C., GUO, W.W. & TREVITHICK, J.R. (1983). Exp. Eye Res. 36, 75-90.

STEWART-DEHAAN, P.J., CREIGHTON, M.O., LARSEN, L.E., [illegible], SANWAL, M., BASKERVILLE, J. & TREVITHICK, J.R. (1985). Exp. Eye Res. 4[illegible], 1-13.

TUKEY, R. (1953). The Problem of Multiple Comparisons. Ditto Princeton University, Princeton, N.J.

WEBBER, M.M., BARNES, F.S., SELTZER, L.A., BOULDIN, T.R. & PRASAD, K.N. (1980). Journal of Ultrastructure Research 71, 321-330.

WEITER, (1971). Ann. N.Y. Acad. Sci. 247, 175-181.

WILLIAMS, R.J., McKEE, A. & FINCH, E.D. (1975). Ann. N.Y. Acad. Sci. 247, 166-174.

Figure 1. Diagram of exposure apparatus showing the wave guide structure and glass holder. For irradiation, the lens is located at the bottom of the central glass tube; buffer, pumped into this tube (shown above the lens) circulates in a water jacket around this tube and then passes out an outlet glass tube at one corner of the jacket. The square metal tube through which the tubes enter is a waveguide, which provides in excess of 100 dB attenuatioin. In addition to the dimensions shown locating the lens, the distance of the lens from the side of the wave guide was 12.86 cm. The lens holder was located 1/4 guide wavelength from the waveguide shorting plate placing the lens holder in the maximum of the electric field. The vertical position of the lens holder was adjusted so that the lens was approximately at the center of waveguide where it may move about (shown by arrows) in the circulating phosphate-buffered saline.

Lens Holder in Waveguide

(Not to scale)

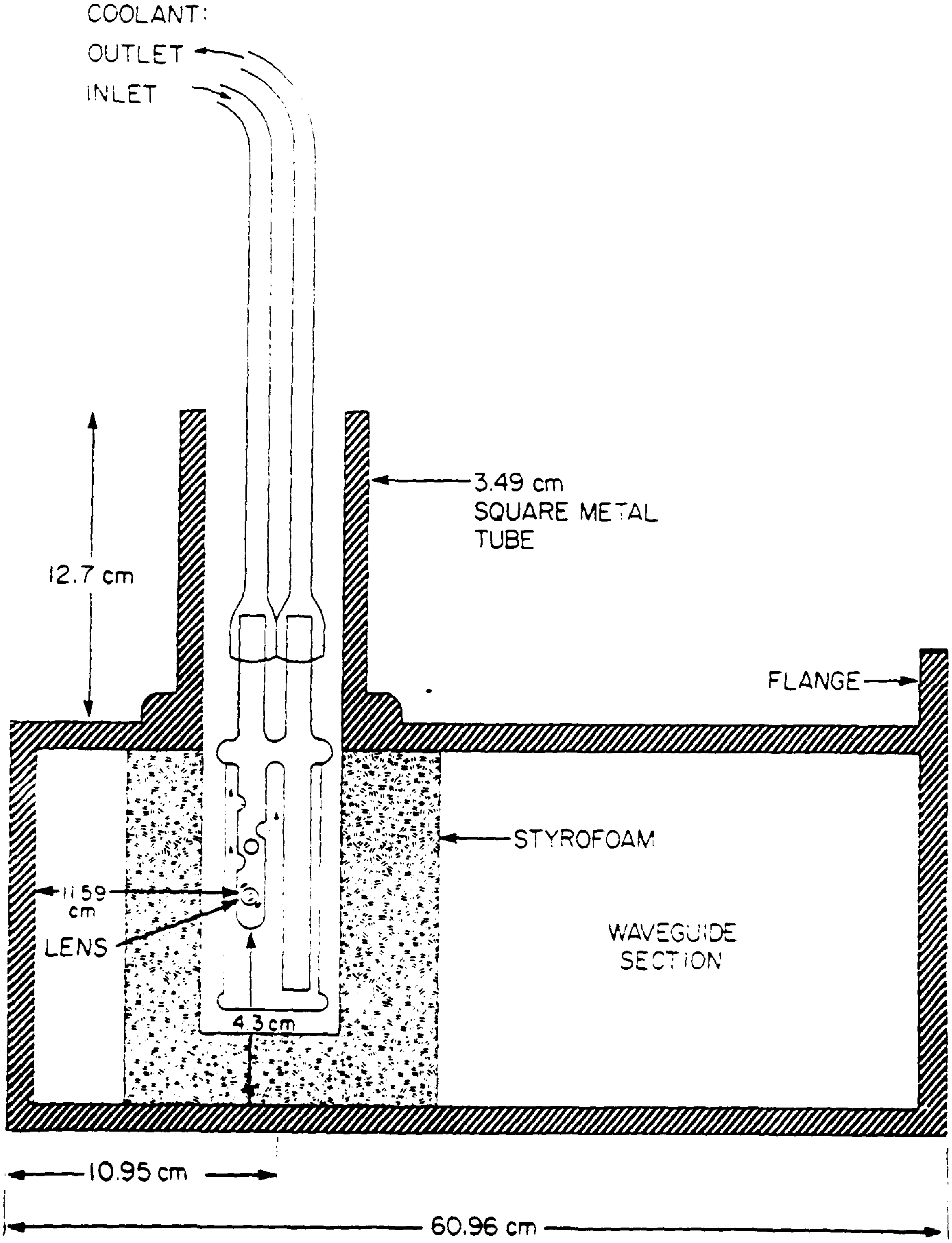
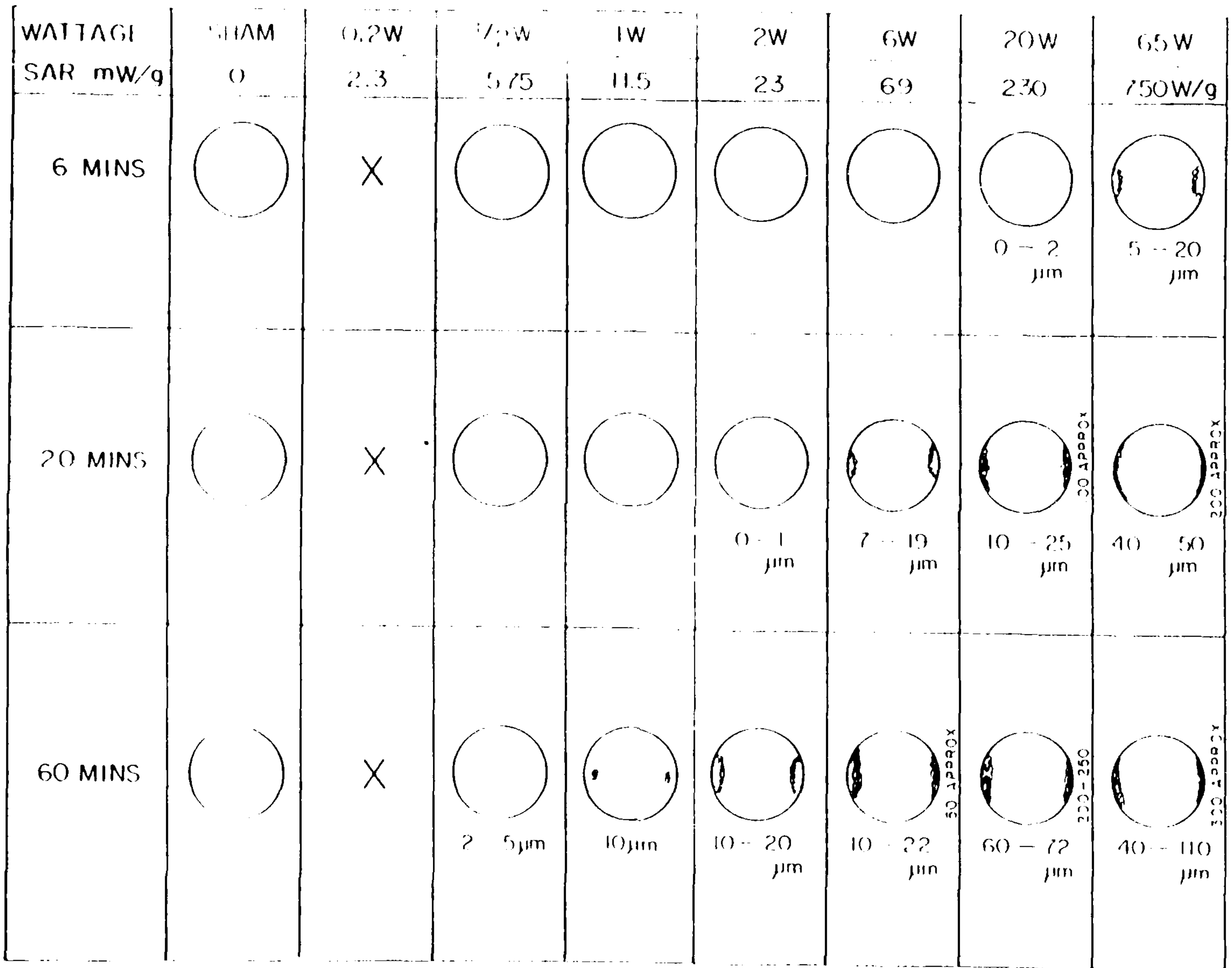


Figure 2. Diagrammatic representation of damage to rat lens observed after exposure to continuous wave (CW) microwaves *in vitro*. The diagrams show the types and extent of damage observed following irradiation with CW microwaves for the times and SAR values indicated. The absence of any additions to the circular lens outline indicates that no damage was observed. The average subcapsula depth of damage in µm is indicated under each lens shown. The lateral extension of visible damage in µm in the immediate subcapsular area is shown (where appropriate) at the right side of each lens.

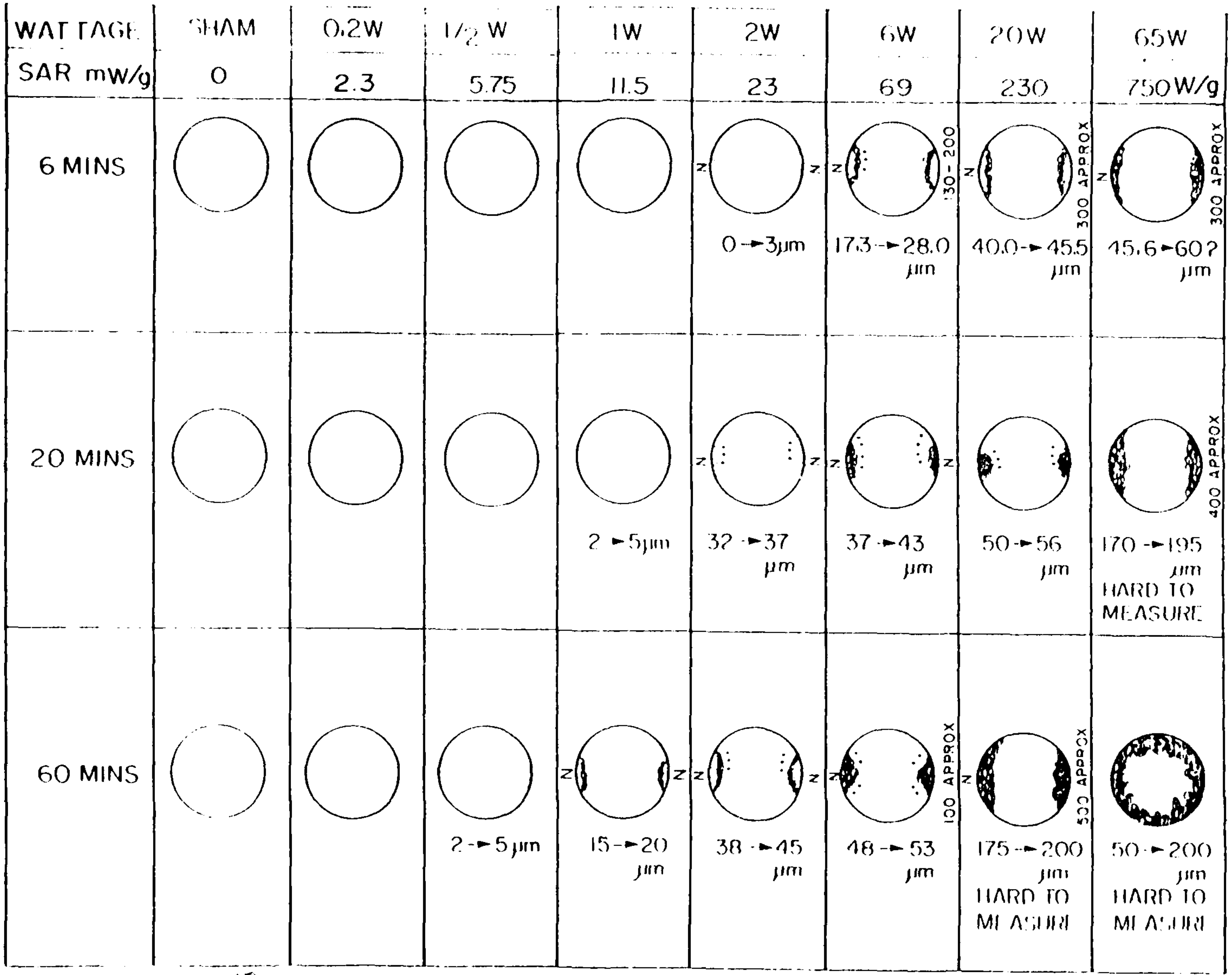
OBSERVED AFTER MICROWAVE EXPOSURE USING CW WAVES



HOLES, FOAM, GRANULAR

Figure 3. Diagrammatic representation of damage to rat lens observed after exposure to pulsed microwaves in vitro. The diagrams illustrate the types and extent of damage observed following irradiation with microwaves for the time and SAR values indicated in the table. The absence of any additions to the circular lens outline indicates that no damage was observed. The average subcapsula depth of damage in μm is indicated under each lens shown. The lateral extension of visible damage in μm in the immediate subcapsula area is shown (where appropriate) at the right side of each lens.

OBSERVED AFTER MICROWAVE EXPOSURE USING PULSED WAVES



HOLES, FOAM, GRANULAR

Figure 4. Average damage observed at the equatorial region of lenses irradiated for 6 min. with Pu or CW microwave irradiation _in vitro_ as described in text. Depth of abnormal morphology holes, spherical bodies, foam and granularity of lens cell surfaces are compared for (a) sham-irradiation, (b) control fixed immediately after dissection without irradiation or sham-irradiation, (c) Pu radiation at 40 mW/g, lowest SAR at which abnormal morphology was detected by SEM for Pu irradiation, compared to (d) CW-irradiation also at 40 mW/g, note absence of abnormal morphology, (e) Pu irradiation at 400 mW/g compared to, (f) CW-irradiation at 400 mW/g, lowest SAR at which abnormal morphology was detected for CW, (g) and (h) 1.3 W/g for (g) Pu and (h) CW to permit comparison of extent of damage at highest SAR used for 6 min irradiation.

Figure 5. Average damage observed at the equatorial region of lenses irradiated for 20 min. with Pu or CW irradiation _in vitro_ as described in text. Depth of abnormal morphology holes, spherical bodies, foam and granularity of lens cell surfaces are compared for (a) sham-irradiation, (b) control fixed immediately after dissection without irradiation or sham-irradiation, (c) Pu radiation at 20 mW/g, lowest SAR at which abnormal morphology was detected by SEM for Pu irradiation for 20 min, (d) CW-irradiation also at 20 mW/g for 20 min: note absence of abnormal morphology, (e) Pu irradiation at 40 mW/g compared to, (f) CW-irradiation also at 40 mW/g, lowest SAR at which abnormal morphology was detected, (d) and (h) 1.3 W/g highest SAR used for (g) Pu and (h) CW to permit comparison of extent of damage at highest SAR used for 20 min irradiation.

Figure 6. Average extent of damage observed at the equatorial region of lenses irradiated for 60 min. with Pu or CW irradiation _in vitro_ as described in text. Depth of abnormal morphology holes, spherical bodies, foam and granularity of lens cell surfaces are compared for (a) sham-irradiation, (b) control fixed immediately after dissection without irradiation or sham-irradiation, (c) Pu radiation at 10 mW/g, lowest SAR at which abnormal morphology was detected by SEM for Pu irradiation for 60 min. (d) CW-irradiation also at 10 mW/g 60 min: note slight (but lesser in extent) abnormal morphology, (e) Pu irradiation at 120 mW/g, an intermediate SAR value t show greater extent of damage as compared to (f) CW-irradiation also at 120 mW/g, (g) and (h) Pu irradiation at SAR 1.3 W/g highest SAR used for (g) Pu and (h) CW irradiation to permit comparison of extent of damage at highest SAR used for 60 min irradiation.

In Vitro Lenticular Damage Diagrammatic Strips

Figure 4 Figure 5 Figure 6

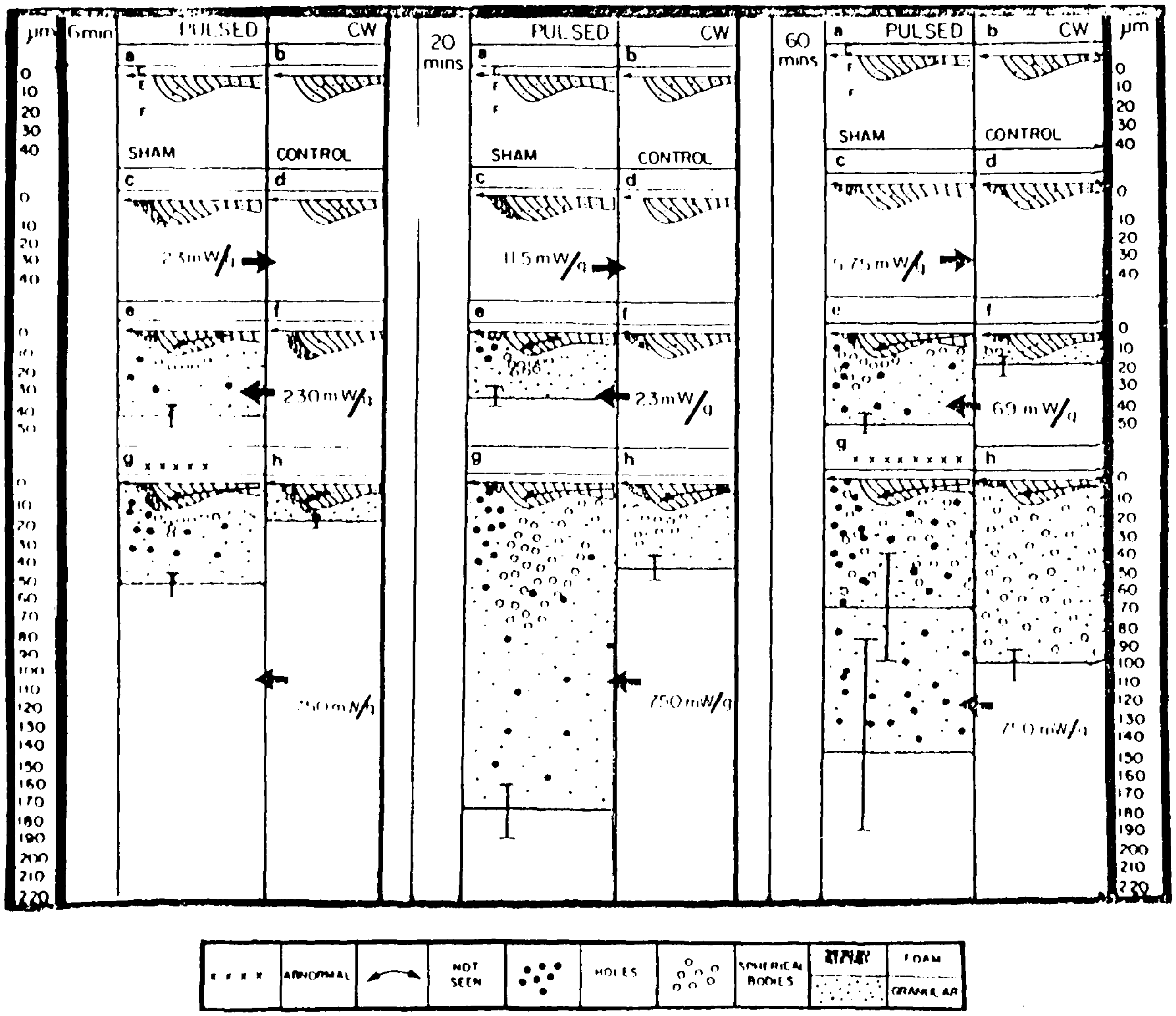


Figure 7. Plot of computer-generated curves using the reciprocal model. illustrating the experimentally observed points and the statistically determined best-fit lines for the equation shown in model 1 (F. 3).

RECIPROCAL MODEL

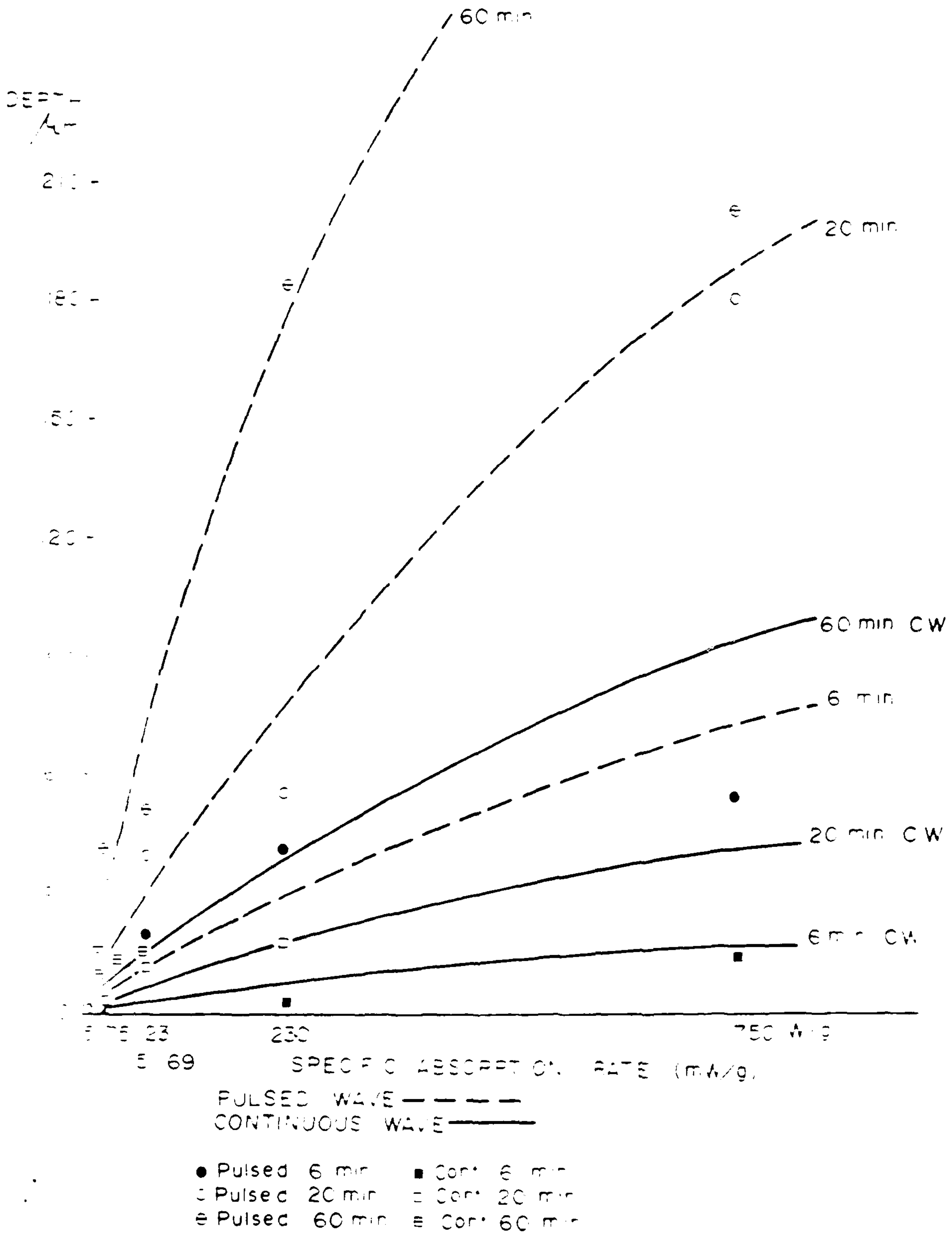


Figure 8. Plot of computer-generated curves using the separate effects model, illustrating the experimentally observed points and the statistically determined best-fit lines for the equation shown in model 2 (F. 3).

SEPARATE EFFECTS MODEL

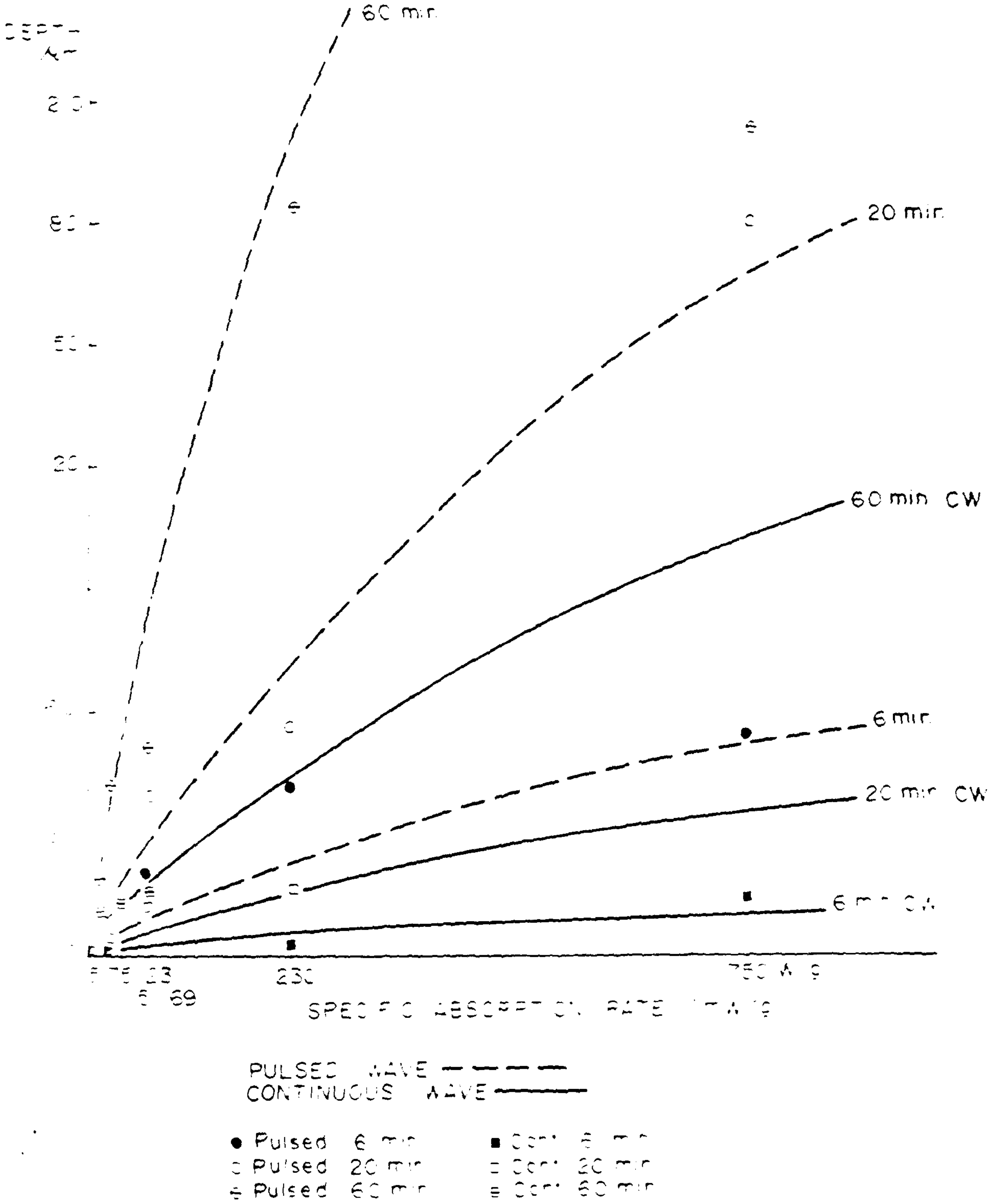


Figure [illegible]. Plot of computer-generated curves for mean depth of damage as a function of an average power or normalized power values, for different peak pulse powers. as outlined in section F. 4.

MEAN DEPTH OF DAMAGE BY AVERAGE POWER AND PEAK POWER

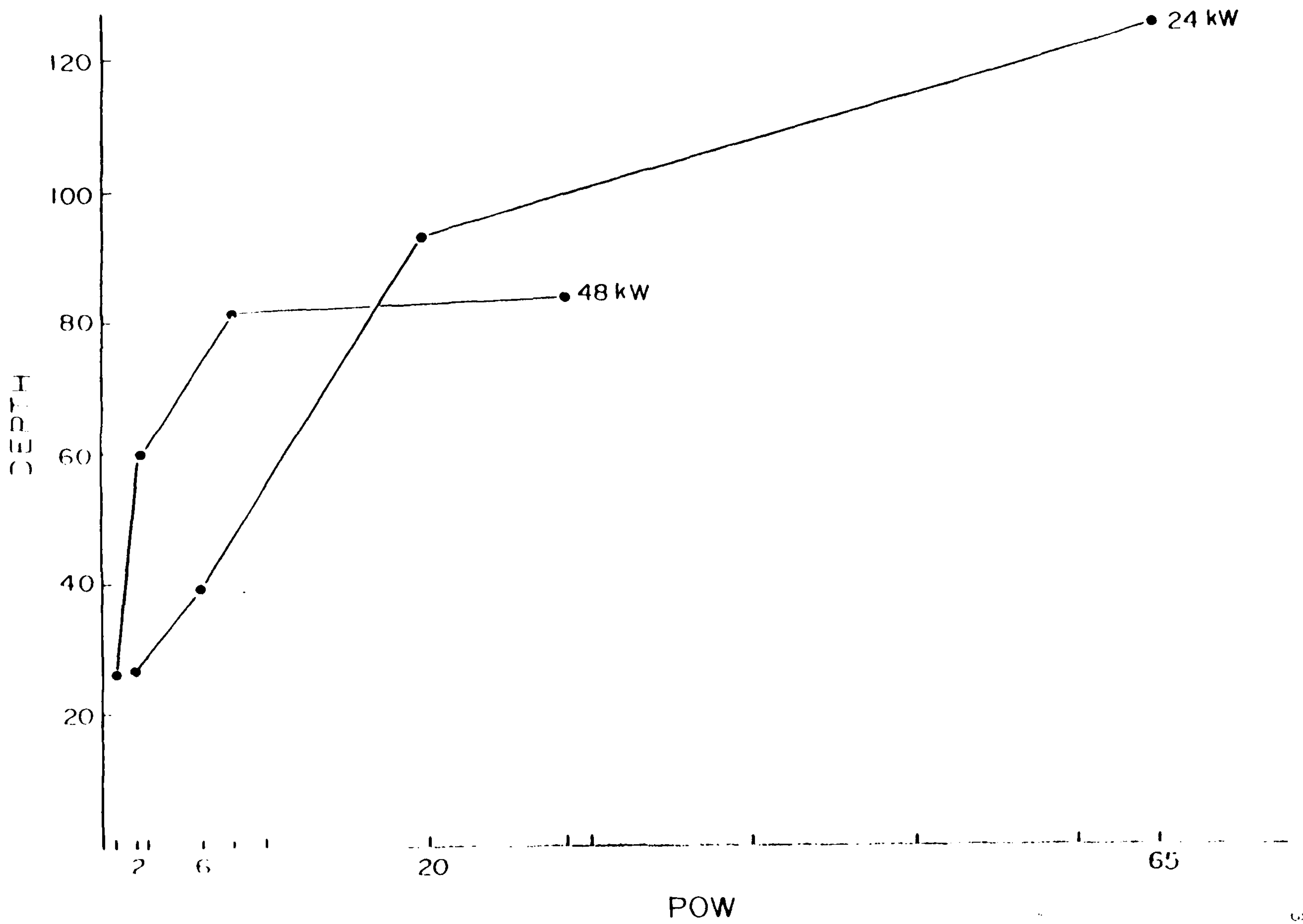


Figure 10. Plot of computer-generated curves of mean depth of damage as a function of average power or normalized power values, for different pulse durations 2, 10 and 20 μseconds, as outlined in section F.4.1.

MEAN DEPTH OF DAMAGE BY AVERAGE POWER AND PULSE WIDTH AT 48 kW

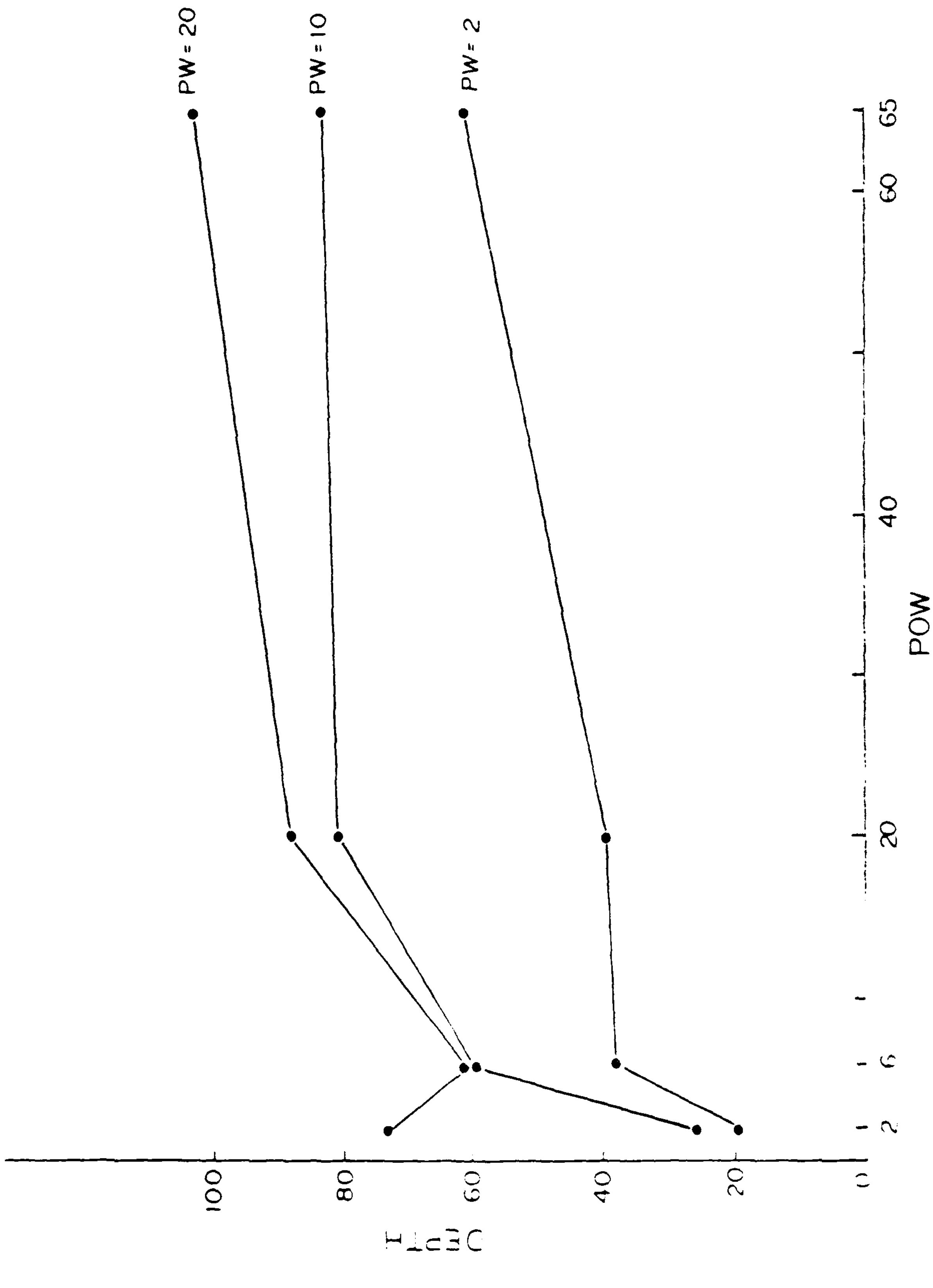


Figure 11. Plot of computer-generate curves of mean depth of damage as a function of pulse width for different duration of exposure to irradiation. at 48 KW peak pulse power as outlined in section F.4.1.

MEAN DEPTH OF DAMAGE BY PULSE WIDTH AND TIME AT 48 kW

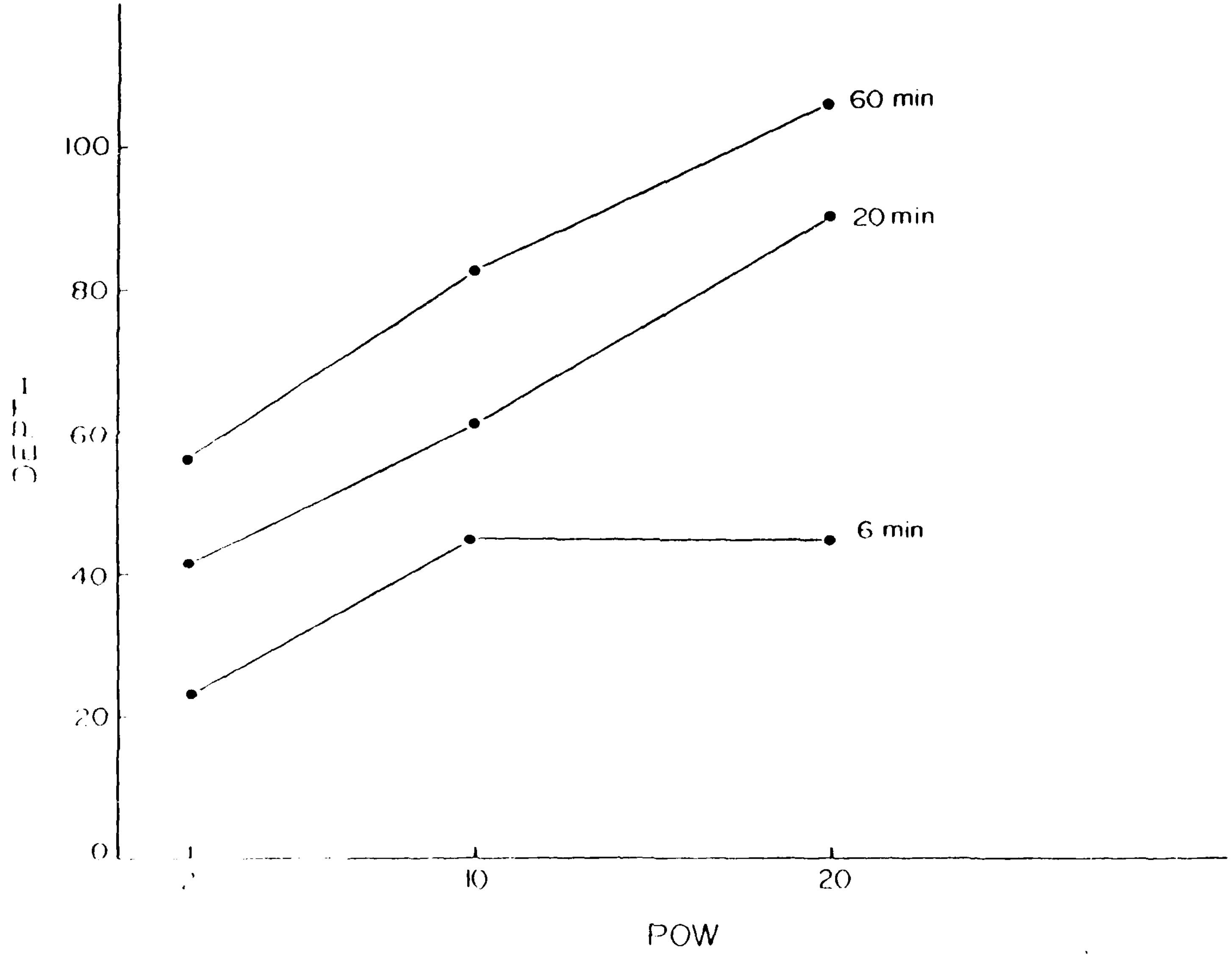


Figure 12. Plot of computer-generated curves of mean depth of damage as a function of average power or normalized power (all at 48 KW peak pulse power) for different durations of exposure to irradiation. The details are indicated in section F.4.1.

MEAN DEPTH OF DAMAGE BY AVERAGE POWER AND TIME AT 48 kW

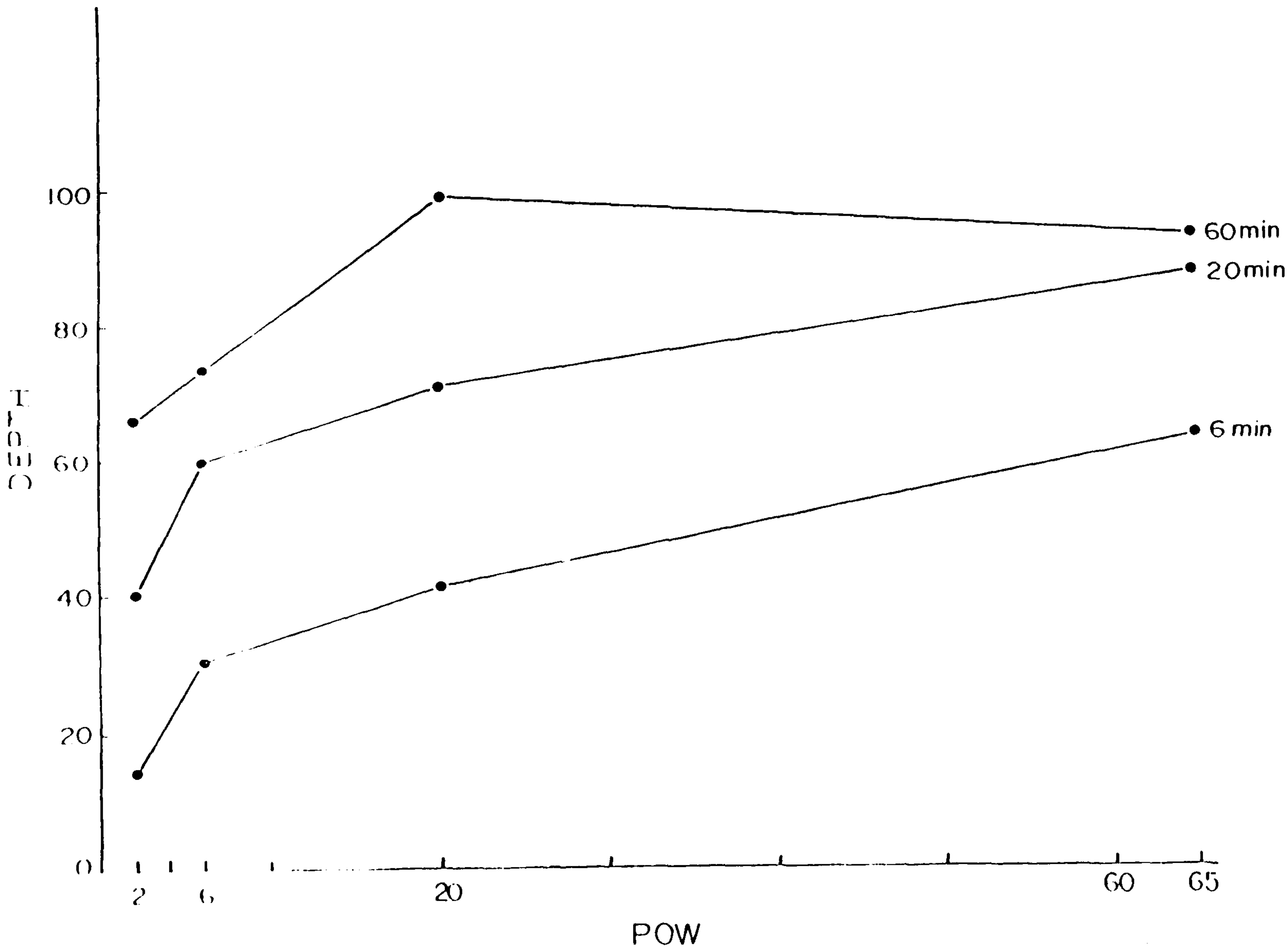


TABLE 1

Threshold of Damage (TD_{50})
(Scanning Electron Microscopy Only)

Time	Type	Normal	Foam	Granulation	Globular Degeneration	Holes in Fiber Cells	Capsular Damage
6 min	Pulsed	up to 6W	6W	6W	20W	6W	65W
	CW	up to 20W	20W	65W	---	---	---
20 min	Pulsed	up to 0.5W	0.5W	2W	2z0W	2W	65W
	CW	up to 0.5W	6W	6W	65W	---	---
60 min	Pulsed	up to 0.5W	---	0.5W	6W	1W	20W
	CW	up to 0.5W	0.5W	6W	65W	65W (1 sample)	---

TABLE 2

Microwave Reciprocity Study (Depth of Damage) for CU Irradiation

Mean Count Sum Std. Dev.	.5 W 1	1 W 2	2 W 3	6 W 4	20 W 5	65 W 6
6 min.	0	0	0	0	.750	14.438
	0	0	4	3	3	4
	0	0	0	0	2.250	57.750
	0	0	0	0	.661	4.175
20 min.	0	0	.813	11.500	17.250	0
	0	0	4	4	4	0
	0	0	3.250	46.000	69.000	0
	0	0	.555	4.818	5.058	0
60 min.	.500	10.688	14.000	15.625	0	0
	3	4	2	4	0	0
	1.500	42.750	28.00	62.500	0	0
	.500	2.839	4.243	2.810	0	0

TABLE 3

Models with Reciprocal and Separate Effects of Exposure Duration (TIME) and Dose Rate (POW) for Continuous Waves.

				R^2
ln(DEP) =	−2.31	+ .87 ln(POW TIME)		.67
±SE	±.47	±.10		
ln(DEP) =	−3.26	+ .82 ln(POW)	= 1.22 ln(TIME)	.76
±SE	±.47	±.09	±.13	

TABLE 4

Test of Reciprocal vs Separate Effects Models for Continuous Waves

	Model (1)		Model (2)	
Source of Variation	df	SS	df	SS
Regression	1	38.94	2	44.51
Residual	37	19.26	36	13.69

$$F = \frac{(44.51 - 38.94)/1}{13.69/36} = 14.64 \; (p < .001)$$

TABLE 5

95% Confidence Intervals for Parameters in Models (1) and (2)

Model	Parameters	Estimate	Confidence Interval
(1)	b_o	.10	.04 to .26
	b_1	.87	.67 to 1.07
(2)	b_o	.04	.01 to .10
	b_1	.82	.64 to 1.00
	b_2	1.22	.96 to 1.47

Note: A 95% confidence interval contains the true value of the estimated parameter with probability .95.

TABLE 6

Models with Reciprocal and Separate Effects of Exposure Duration (TIME) and Dose Rate (POW) for Pulsed and Continuous Waves

$\underline{R^2}$ <u>Reciprocal Effects</u>

.84 $\ln(\text{DEP}) = -1.91 = .78 \ln(\text{POW X TIME})$ for continuous

$= -.36 + .78 \ln(\text{POW X TIME})$ for pulsed

SE $\pm .05$

effect of radiation type: $\ln(b_1) = 1.55 \pm .14$

$b_1 = 4.71$ (pulsed is 4.71xCW)

$\underline{R^2}$ <u>Separate Effects</u>

.85 $\ln(\text{DEP}) = -2.41 + .74 \ln(\text{POW}) + .97 \ln(\text{TIME})$ for continuous

$= -.87 + .74 \ln(\text{POW}) + .97 \ln(\text{TIME})$ for pulsed

effect of radiation type: $\ln(b_1) = 1.54 \pm .13$

$b_1 = 4.66$

TABLE 7

Test of Reciprocal vs Separate Effects Models for

Combined Data for Continuous and Pulsed Waves

	Model (3)		Model (4)	
Source of Variation	df	SS	df	SS
Regression	2	200.84	3	204.90
Residual	92	39.61	91	35.56

$$F = \frac{204.90 - 200.84)/1}{35.56/91} \approx 10.41 \quad (p < .005)$$

TABLE 8

Microwave Reciprocity Study (Depth of Damage for Pulsed Irradiation)

Mean Count Sum Std. Dev.	.5 W 1	1 W 2	2 W 3	6 W 4	20 W 5	65 W 6
6 min.	0	0	.625	21.433	42.475	51.560
	0	0	4	3	4	5
	0	0	2.500	64.300	169.900	257.800
	0	0	.750	4.704	1.394	6.140
20 min.	4.063	0	33.500	39.813	54.638	180.000
	4	0	4	4	4	4
	16.250	0	134.000	159.250	218.550	720.000
	4.195	0	2.656	2.095	1.775	8.414
60 min.	0	17.750	41.620	51.112	185.625	203.750
	0	5	5	4	4	2
	0	88.750	208.100	204.450	742.500	407.500
	0	3.992	1.702	1.288	8.985	1.768

TABLE 9

95% Confidence Intervals for parameters in Models (3) and (4)

Model	Parameter	Estimate	Confidence Interval
(3)	b_o	.15	.09 to .24
	b_1	4.71	3.56 to 6.23
	b_2	.78	.69 to .88
(4)	b_o	.09	.05 to .16
	b_1	4.66	3.60 to 6.11
	b_2	.74	.65 to .84
	b_3	.97	.82 to 1.12

TABLE 10

MICROWAVE RECIPROCITY STUDY (DEPTH OF DAMAGE) 85/04/21. 12.55.20.

FILE NONAME (CREATION DATE = 85/04/21.)

* * * * * * * * * * * * * * * * * * C R O S S - - B R E A K D O W N O F * * * * * * * * * * * * *

TIME DURATION OF EXPOSURE BY POW AVERAGE POWER IN WATTS

CONTROLLING FOR..

PP PEAK POWER VALUE.. 2 24 KW

* * * * * * * * * *

VARIABLE AVERAGED.. DEP

* P

TIME	POW: MEAN / COUNT / SUM / STD DEV	2 WATTS 3	6 WATTS 4	20 WATTS 5	55 WATTS 6	ROW TOTAL
6 MINUTES	1	.525	21.433	42.475	51.560	30.904
		4	3	4	5	16
		2.500	64.300	169.900	257.800	494.500
		.750	4.704	1.794	6.140	21.394
20 MINUTES	2	33.500	38.813	54.638	180.000	76.993
		4	4	4	4	16
		134.000	155.250	218.550	720.000	1231.800
		2.656	2.095	1.775	9.416	62.072
60 MINUTES	3	41.620	51.112	185.625	203.750	104.170
		5	4	4	2	15
		208.100	204.450	742.500	407.500	1562.550
		1.702	1.288	8.785	1.768	74.392
TOTAL COLUMN		26.508	38.909	94.246	125.936	69.926
		13	11	12	11	47
		344.600	428.000	1130.950	1385.300	3286.850
		10.375	12.560	67.960	71.991	63.384

TABLE 11

MICROWAVE RECIPROCITY STUDY (DEPTH OF DAMAGE) 85/04/21. 12.55.29.

FILE NONAME (CREATION DATE = 85/04/21.)

* C R O S S---B R E A K D O W N O F * * * * * * * * * * * * *

TIME DURATION OF EXPOSURE BY POW AVERAGE POWER IN WATTS
CONTROLLING FOR..
PP PEAK POWER VALUE.. 3 40 KW
* * * * * * * * * *
VARIABLE AVERAGED.. DEP
* P

TIME	POW (MEAN / COUNT / SUM / STD DEV)	2 WATTS (3)	5 WATTS (4)	20 WATTS (5)	55 WATTS (6)	ROW TOTAL
5 MINUTES	1	9.775	45.750	43.750	76.600	45.467
		4	4	4	4	16
		39.100	183.000	199.000	306.400	727.500
		4.862	22.456	7.377	38.056	31.771
20 MINUTES	2	17.625	60.000	75.625	91.875	61.367
		4	3	4	4	15
		70.500	180.000	302.500	367.500	920.500
		6.028	5.614	17.452	9.331	31.127
60 MINUTES	3	51.625	75.000	133.667	82.500	82.500
		4	4	3	1	12
		206.500	300.000	401.000	82.500	990.000
		6.625	10.206	5.132	0	33.560
TOTAL COLUMN		26.342	60.273	82.045	84.044	61.349
		12	11	11	9	43
		316.100	663.000	902.500	756.400	2638.000
		19.703	13.037	36.589	25.193	34.675

Table 12: P-Values and Means From ANCOVA of Depth of Damage

Factor	P-Value	
NPOW (Covariate)	.001	(Regression Coefficient=1.4)
PEAK POWER (PP)	.02	
TIME	.001	
PPxTIME	.01	

PP	Means	Adjusted Means
24	70.0	57.8
48	61.3	74.7

TIME	Means	Adjusted Means
6	38.2	33.3
20	69.4	67.3
60	94.5	102.8

```
END OF FILE ON FILE INPUT
AFTER READING    192 CASES FROM SUBFILE NONAME

MICROWAVE RECIPROCITY STUDY (DEPTH OF DAMAGE)                    86/02/03.    16.04.59.

FILE    NONAME    (CREATION DATE =  86/02/03.)

* * * * * * * * * * * * * * * * * *   C R O S S---B R E A K D O W N   O F   * * * * * * * * * * * * *
    TIME        DURATION OF EXPOSURE                         BY   POW        AVERAGE POWER IN WATTS
CONTROLLING FOR..
    PP          PEAK POWER                                   VALUE..      2   24 KW
* * * * * * * * * *
VARIABLE AVERAGED..  DEP
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *   PA(
```

TIME	MEAN / COUNT / SUM / STD DEV	POW 2 WATTS (3)	6 WATTS (4)	20 WATTS (5)	65 WATTS (6)	ROW TOTAL
6 MINUTES	1	9.000 1 9.000 0	37.500 1 37.500 0	49.000 1 49.000 0	80.500 1 80.500 0	44.000 4 176.000 29.578
20 MINUTES	2	46.000 1 46.000 0	43.000 1 43.000 0	67.500 1 67.500 0	135.000 1 135.000 0	72.875 4 291.500 42.830
60 MINUTES	3	67.500 1 67.500 0	80.500 1 80.500 0	180.000 1 180.000 0	168.000 1 168.000 0	124.000 4 496.000 58.185
TOTAL COLUMN		40.333 3 122.500 29.590	53.667 3 161.000 23.400	98.833 3 296.500 70.898	127.833 3 383.500 44.188	80.292 12 963.500 53.440

TABLE 13

MICROWAVE RECIPROCITY STUDY (DEPTH OF DAMAGE) 86/04/19. 11.45.29.

FILE NONAME (CREATION DATE = 86/04/19.)

* C R O S S - - - B R E A K D O W N O F * * * * * * * * * * * * *

TIME DURATION OF EXPOSURE BY POW AVERAGE POWER IN WATTS
CONTROLLING FOR..
PW PULSE WIDTH VALUE.. 2 2 US
* * * * * * * * * * *
VARIABLE AVERAGED.. DEP
* *

TIME	POW: MEAN / COUNT / SUM / STD DEV	2 WATTS 3	6 WATTS 4	20 WATTS 5	55 WATTS 6	ROW TOTAL
5 MINUTES	1	12.637	20.837	19.725	41.425	23.655
		4	4	4	4	16
		50.550	83.350	78.900	165.700	378.500
		3.613	2.108	4.315	4.342	11.563
20 MINUTES	2	22.338	31.000	39.013	68.875	42.454
		4	1	4	4	13
		89.350	31.000	156.050	275.500	551.900
		8.173	0	6.549	17.159	21.983
60 MINUTES	3	23.500	56.250	62.500	77.000	56.900
		3	4	4	4	15
		70.500	225.000	250.000	308.000	853.500
		9.124	5.107	9.398	17.517	21.535
TOTAL COLUMN		19.127	37.706	40.412	62.433	40.543
		11	9	12	12	44
		210.400	339.350	484.950	749.200	1783.900
		9.205	10.201	19.290	20.539	23.097

TABLE 14

MICROWAVE RECIPROCITY STUDY (DEPTH OF DAMAGE) 86/04/18. 11.45.28.

FILE NONAME (CREATION DATE = 86/04/18.)

* * * * * * * * * * * * * * * * * C R O S S - - B R E A K D O W N O F * * * * * * * * * * * *

TIME DURATION OF EXPOSURE BY POW AVERAGE POWER IN WATTS
CONTROLLING FOR..
PW PULSE WIDTH VALUE.. 3 10 US
* * * * * * * * * *
VARIABLE AVERAGED.. DEP
* *

TIME	POW: MEAN / COUNT / SUM / STD DEV	2 WATTS (3)	6 WATTS (4)	20 WATTS (5)	55 WATTS (6)	ROW TOTAL
6 MINUTES	1	8.775	65.750	49.750	76.600	45.469
		4	4	4	4	15
		35.100	263.000	199.000	306.400	722.500
		4.862	22.456	7.377	39.056	31.771
20 MINUTES	2	17.625	60.000	75.625	91.875	61.367
		4	3	4	4	15
		70.500	180.000	302.500	367.500	920.500
		6.028	6.614	17.452	9.331	31.12?
60 MINUTES	3	51.625	75.000	133.667	82.500	82.500
		4	4	3	1	12
		206.500	300.000	401.000	82.500	990.000
		5.625	10.206	5.132	0	33.560
TOTAL COLUMN		26.342	60.273	82.045	84.044	61.349
		12	11	11	9	43
		315.100	663.000	902.500	756.400	2638.000
		10.703	19.037	35.599	25.133	34.675

TABLE 15

MICROWAVE RECIPROCITY STUDY (DEPTH OF DAMAGE) 86/04/18. 11.45.28.

FILE NONAME (CREATION DATE = 86/04/18.)

* * * * * * * * * * * * * * * * * * C R O S S - - - B R E A K D O W N O F * * * * * * * * * * * * *

TIME DURATION OF EXPOSURE BY POW AVERAGE POWER IN WATTS
CONTROLLING FOR..
PW PULSE WIDTH VALUE.. 4 20 US

VARIABLE AVERAGED.. DEP

TIME	POW: MEAN / COUNT / SUM / STD DEV	2 WATTS (3)	6 WATTS (4)	20 WATTS (5)	55 WATTS (6)	ROW TOTAL
6 MINUTES	1	21.375	25.138	56.625	76.625	44.941
		4	4	4	4	16
		85.500	100.550	226.500	306.500	719.050
		6.559	6.267	20.347	8.528	25.949
20 MINUTES	2	82.875	69.500	99.875	103.875	90.267
		4	3	4	4	15
		331.500	208.500	399.500	415.500	1354.000
		27.285	3.041	28.584	24.496	25.630
60 MINUTES	4	106.900	88.875	109.500	167.500	106.821
		5	4	4	1	14
		534.500	355.500	438.000	167.500	1495.500
		28.230	9.750	34.414	0	30.414
TOTAL COLUMN		73.115	60.414	88.667	98.833	79.301
		13	11	12	9	45
		950.500	664.550	1064.000	889.500	3568.550
		43.230	29.811	35.104	33.181	37.714

TABLE 16

Table 17: P-Values From ANOVA on 48 Kw Data

Factor	P-Value
PULSE WIDTH (PW)	.001
POWER (POW)	.001
TIME	.001
PWxPOW	.001
PWxTIME	.001
POWxTIME	.79
PWxPOWxTIME	.007

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